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Data Sheet

PDGFRα (D842V) Assay Kit

Catalog # 79828
96 Reactions

DESCRIPTION: Platelet-derived growth factor receptor A or PDGFRα has been implicated in regulation of cell growth and survival, apoptosis, and differentiation. It has been identified as a potential target in eosinophilic leukemia cancer, inflammatory breast cancer, and gastrointestinal stromal tumors. The D842V is one of the most predominant of the D842 mutations, which are related to gastrointestinal stromal tumors and are associated with resistance to tyrosine kinase inhibitors. The *PDGFRα (D842V) Assay Kit* is designed to measure PDGFRα (D842V) activity for screening and profiling applications using ADP-Glo® Kinase Assay as a detection reagent. The *PDGFRα (D842V) Assay Kit* comes in a convenient 96-well format, with enough purified recombinant PDGFRα (D842V), Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1), ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
79633	PDGFRα (D842V), GST-Tag	2 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
40217	Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
ADP-Glo Kinase Assay (Promega #V6930)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE:
Evans, E., *et al.* A Precision Therapy Against Cancers Driven by KIT/PDGFRA Mutations; 2017, *Science Translational Medicine* Nov; **9(414):** 1690.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**)

2) Prepare the master mixture (12.5 µl per well): N wells x (3 µl **5x Kinase assay buffer** + 0.5 µl **ATP (500 µM)** + 0.5 µl **Protein Tyrosine Kinase Substrate (Poly-Glu,Tyr 4:1)** + 8.5 µl distilled water). Add 12.5 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	3 µl	3 µl	3 µl
ATP (500 µM)	0.5 µl	0.5 µl	0.5 µl
Poly-Glu,Tyr(10 mg/ml)	0.5 µl	0.5 µl	0.5 µl
Water	8.5 µl	8.5 µl	8.5 µl
Test Inhibitor	–	2.5 µl	–
10% DMSO in Water (inhibitor buffer)	2.5 µl	–	2.5 µl
1x Kinase buffer	–	–	10 µl
PDGFRα (D842V) (1.5 ng/µl)	10 µl	10 µl	–
Total	25 µl	25 µl	25 µl

3) Add 2.5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 2.5 µl of 10% DMSO in water (Inhibitor buffer). For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 2.5 µl of the 100 µM solution into the 25 µl assay to make a 1% DMSO concentration in the final reaction mixture. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity.*

4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 10 µl of **1x Kinase assay buffer**.

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6) Thaw **PDGFRα (D842V), GST-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **PDGFRα (D842V), GST-Tag** required for the assay and dilute enzyme to 1.5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PDGFRα (D842V), GST-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 10 µl of diluted **PDGFRα (D842V), GST-Tag** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw ADP-Glo reagent.

9) After the 45 minute reaction, add 25 µl of ADP-Glo reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

10) Thaw Kinase Detection reagent.

11) After the 45 minute incubation, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes

12) Measure luminescence using the microplate reader. “Blank” value is subtracted from all readings.

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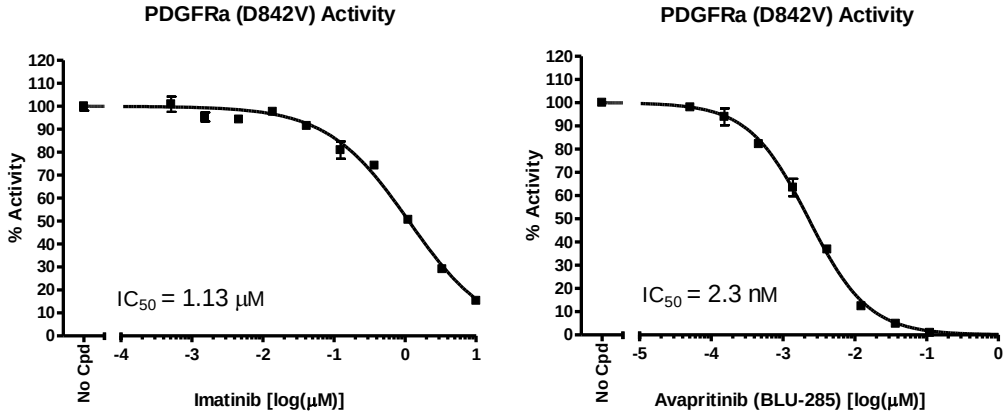
Example of Assay Results:



Inhibition of PDGFRα (D842V), GST-Tag by Imatinib and Avapritinib, measured using the PDGFRα (D842V) assay kit (BPS Bioscience #79828). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
PDGFRα (D842I), GST-Tag	#100202	10 µg
PDGFRα (D842Y), GST-Tag	#100201	10 µg
PDGFRα (D842V), GST-Tag	#79633	10 µg
PDGFRα GST-Tag	#40261	10 µg
PDGFRα, Mouse, GST-Tag	#40260	10 µg
PDGFRα (D842I) Assay Kit	#79761	96 rxns.
PDGFRα (D842Y) Assay Kit	#79760	96 rxns.
PDGFRβ, His-tag	#40263	10 µg
ATP (500 µM)	#79686	200 µl
Kinase Buffer 1	#79334	10 ml
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	#40217	1 mg

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